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COMPOSITION OF INDICATOR BACTERIA IN INDUSTRIAL AND BACKYARD CHICKEN FARMING

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Keywords: *microflora, chicken, zoonosis, housing conditions.*

Introduction. *The intestinal microbiocenosis is the most complex and important biotope of the body formed in the process of individual development.*

Material and methods. *The study was conducted on groups of 20-25-day-old chicks. The first group was housed under standard vivarium conditions with artificially maintained optimal climatic parameters. The second group was raised in a rural homestead in the Kyiv region, on pasture with access to water, and fed twice daily with a blend of grains supplemented with kitchen wastes. Samples of chicken droppings (10 per group) were analyzed according to current international ISO standards using certified nutrient media and equipment.*

Results. *Escherichia coli, Klebsiella spp., Enterococcus spp. were isolated from 100% of samples from chickens kept in simulated conditions of an industrial poultry house, and Pseudomonas aeruginosa was isolated from 70% of samples. E. coli and Enterococcus spp. were isolated from free-range chickens in 100% of cases. The analysis revealed that in pasture-raised chickens, Klebsiella spp. and P. aeruginosa were absent from the litter, with significantly higher levels of normal microflora (Enterococcus spp.).*

Conclusions. *Backyard-raised chickens showed no pathogenic zoonotic bacteria, in contrast to those raised under controlled conditions with optimal climate and standard diets.*

Cuvinte-cheie: *microfloră, pui, zoonoze, condiții de trai.*

RAPORTUL INDICATORILOR BACTERIENI LA PUII CRESCUȚI ÎN CONDIȚII INDUSTRIALE ȘI LA PUII CRESCUȚI ÎN AER LIBER

Introducere. *Microbiocenoza intestinală, formată în procesul dezvoltării individuale, reprezintă cel mai complex și cel mai important biotop al organismului.*

Material și metode. *Au fost formate două loturi de studiu, constituite din pui de 20-25 de zile. Primul grup a fost ținut în condiții standard de vivarium, cu asigurarea artificială a condițiilor climatice optime. Al doilea grup a fost ținut într-o gospodărie sătească din regiunea Kiev, pe pășune, având acces liber la apă și la hrană (un amestec de cereale cu adaos de deșeuri de bucătărie) de două ori pe zi . Probele de excremente de pui (10 per grup) au fost analizate în conformitate cu standardele internaționale ISO actuale, folosind medii și echipamente nutritive certificate.*

Rezultate. *În probele recoltate de la puii care au fost ținuți în condiții simulate, într-un adăpost industrial de păsări, în proporție de 100% au fost izolate Escherichia coli, Klebsiella spp. și Enterococcus spp., iar Pseudomonas aeruginosa a fost identificată în 70% de probe. La puii crescuți în aer liber, E. coli și Enterococcus spp. au fost izolate, de asemenea, în 100% dintre cazuri. Analiza rezultatelor a arătat că în așternutul puilor ținuți „pe pășune" nu s-au depistat Klebsiella spp. și P. aeruginosa, determinându-se, în același timp, un conținut semnificativ mai mare de reprezentanți ai microflorei normale (Enterococcus spp.).*

Concluzii. *La găinile crescute în condiții de curte s-a constatat absența bacteriilor zoonotice cu potențial patogen, spre deosebire de puii ținuți în condiții climatice optime, asigurate artificial, cu o dietă standard.*

INTRODUCTION

The gut microbiome plays a crucial role in various aspects of chicken physiology, including growth, feed conversion efficiency, immune system development, homeostasis maintenance, metabolic regulation, and resistance to pathogens (1, 2, 3). Recent studies have highlighted the significant impact of environmental factors on the gut microbiota (4).

During the formation of eggs in the oviduct and their passage through the reproductive tract, bacterial contamination can occur. Moreover, the embryonic stage of chickens already harbors diverse microorganisms within their digestive tracts (5). The environment of incubation cabinets significantly affects the formation of the microbiocenosis of the digestive tract of chickens. Furthermore, microbial contamination of eggshells serves as a potential source of bacteria for chickens, often occurring immediately after laying due to contact with contaminated processing equipment (6, 7). The microbial composition introduced into the body post-hatching varies depending on numerous factors, including production practices, husbandry technologies, feeding systems, etc. (8).

From the moment chickens hatch, their digestive tract becomes populated by a variety of environmental microorganisms such as *E. coli*, bacteria from genera like *Lactobacillus, Bacillus, Streptococcus, Bifidobacterium*, etc. (9). The composition of this intestinal microbiota is influenced by numerous factors, including diet, climate, and environmental conditions (10, 11), as well as other factors (12, 13). Considering the above, it is important to study how the housing environment impacts the species composition of poultry microbiota to optimize biosecurity protocols.

Microbial populations vary significantly across different segments of birds' digestive systems. For instance, concentrations range from 10^3-10^4 CFU/g in the stomach, 10^2-10^3 CFU/g in the glandular and muscular stomachs, and 10^3 CFU/g in the duodenum. The most microorganisms are found in the end sections of the small intestines, cecum, and rectum, where it ranges from 10^7 to 10^9 CFU/g, respectively.

The findings from recent research (14, 15) suggest that *Lactobacillus* spp., *Bifidobacterium* spp., *Klebsiella pneumoniae, Klebsiella ozaenae, Escherichia coli, Escherichia fergusonii, Enterobacter aerogenes, Eubacterium* spp., *Pseudomonas aeruginosa, Micrococcus luteus, Staphylococcus lentus*, and *Sarcina* spp. are indicative of the body's homeostasis in specific regions of the digestive tract.

Lactobacillus spp. typically colonize the digestive tract (16, 17), inhabiting various regions from the oral cavity to the rectum. These bacteria produce lactase, lysozyme, lactic acid, hydrogen peroxide, and various antibiotic-like compounds (such as lactocidin, lactocin, reuterin, plantaricin, lactolin, acidophilin) that inhibit the growth of putrefactive opportunistic microbes and pathogens causing acute intestinal infections. Upon interacting with enterocytes, they stimulate the bird's defense mechanisms, maintain colon acidity at pH 5.5-5.6, stimulate the phagocytic activity in neutrophils and macrophages, promote immunoglobulin synthesis and interferon formation, and participate in proteolysis and lactose metabolism processes.

Analysis of cecal microbiota using molecular approaches has identified bacterial populations of more than 600 species from more than 100 genera. However, many of these bacteria remain unclassified species or genera (18, 19). Previous research (20) established gram-positive cocci, *Clostridium* spp., *E. coli, Lactobacillus* spp., *Streptococcus* spp., *Acinetobacter* and *Acidobacteri*a which dominate the microbiota of the small intestine, while *Bacteroides,* whereas *Bacteroides* and *Clostridium* predominate in the cecum (21).

Maintaining a healthy gut is intricately linked to a balanced interaction between the immune system and the endogenous microbiota (22). A healthy avian intestine, as a rule, participates in the maintenance of intestinal homeostasis with the help of a complex network of cells and their secreted soluble products (23). The intestinal microbiota plays a crucial role in modulating the host's immune system, influencing organ development, and regulating host metabolism (24). Mucosal immune responses to resident intestinal microbiota can distinguish commensal from pathogenic bacteria (25). Gut microbiota is also involved in modulating B-cell response and immunoglobulin A (IgA) production. IgA plays an important role in regulating the composition of the intestinal microbiota through specific binding to bacterial epitopes. Thus, according to Aruwa C.E. et al. (26), maintaining intestinal health is para-

mount for the efficient and sustainable functioning of the avian digestive tract.

This study aimed to investigate the species composition of indicator opportunistic bacteria in the droppings of clinically healthy chickens that were kept under different environmental conditions.

MATERIAL AND METHODS

The study was carried out on groups of 20-25-day-old chicks, which were kept in different conditions. The first group of chickens (cross Cobb 500) was raised within vivarium conditions in NUBIP of Ukraine, housed in a standard KR 108 collapsible cage designed for laying hens and broilers that received a standard artificial diet under optimal climatic conditions (30±3°C, humidity 55±5%).

The second group of chicks was raised in a homestead located in the village of Gatne, Fastiv district, Kyiv region, within a free-range setting, having unrestricted access to water, being fed a diet of home-grown wheat and corn, which were locally sourced, chopped, steamed, and supplemented with kitchen wastes.

Throughout the study, all protocols adhered strictly to the guidelines outlined in EU Directive 2010/63/EU concerning the ethical treatment of animals used for scientific research.

Samples of chicken droppings (10 samples per group) from clinically healthy chickens were delivered in a thermal container at a temperature of 2-8°C. The research was conducted at the scientific laboratory of the Faculty of Veterinary Medicine using certified nutrient media and equipment, in accordance with the following regulatory standards.

Preparation of test samples, initial suspension and tenfold dilutions for microbiological examination was carried out in accordance with ISO 6887-1:2017 "Microbiology of the food chain Preparation of test samples, initial suspension and decimal dilutions for microbiological examination Part 1: General rules for the preparation of the initial suspension and decimal dilutions".

Isolation and determination of the most probable number (MPN) of enterobacteria, *E. coli, Klebsiella* spp., were carried out in accordance with ISO 21528-1:2017: Microbiology of the food chain – Horizontal method for the detection and enumeration of *Enterobacteriaceae* – Part 1: Detection of *Enterobacteriaceae"*.

Isolation and determination of the most probable number (MPN) of enterococci was carried out in accordance with DSTU 8534:2015 "Food products. A method for detecting and determining the number of enterococci".

The technique of the most likely number (MPN) involves the use of the MPN table with a 95% confidence interval and the corresponding formula for calculating the number of microorganisms.

Isolation and determination of *P. aeruginosa* was carried out in accordance with the "Methodological recommendations. Detection and identification of *P. aeruginosa* in environmental objects (food products, water, wastewater)".

Isolation and identification of *Salmonella* spp. carried out in accordance with ISO 6579-1:2017 "Microbiology of the food chain horizontal method for the detection, enumeration and serotyping of *Salmonella* part 1: detection of *Salmonella* spp.".

Isolation and identification of *Listeria* spp./*Listeria monocytogenes* was carried out in accordance with ISO 11290-1:2017 "Microbiology of the food chain Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. Part 1: Detection method".

Isolation and identification of *Yersinia enterocolitica* was carried out in accordance with ISO 10273:2017 "Microbiology of the food chain Horizontal method for the detection of pathogenic *Yersinia enterocolitica*".

RESULTS

Based on bacteriological studies of samples obtained from chicken groups 1 and 2, no *Yersinia* spp., *Salmonella* spp., *or Listeria* spp./*Listeria monocytogenes* were isolated.

In group 2 samples, *Enterococcus* spp. *and E. coli* were isolated in 10 cultures each, while *Klebsiella* spp. and *P. aeruginosa* were not found in chicken litter samples from this group.

Isolated cultures of *E. coli* are gram-negative motile rods, catalase-positive, oxidase-negative, facultative anaerobes that ferment glucose and lactose with the formation of acid and gas. In the liquid medium of meat peptone broth, daily cultures of *E. coli* formed a uniform turbidity with a small amount of white amorphous sediment that easily

broke up upon agitation.

On dense meat peptone agar (MPA), *E. coli* cultures formed S-shaped colonies measuring 2-4 mm in diameter, appearing transparent, delicate, and grayish in color. On XLD agar (xylose-lysine deoxycholate agar) (HiMedia), *E. coli* colonies exhibited a yellow coloration, with the surrounding medium changing from red to yellow. Additionally, *E. coli* cultures formed blue colonies on Salmonella differential agar (M1078 Raj Hansa medium) (HiMedia).

E. coli cultures did not grow on the selective differential diagnostic medium bismuth-sulfite agar M1031 (HiMedia). On the chromogenic medium HiCrome *E. coli* Agar M 12951 (HiMedia), isolated *E. coli* cultures formed distinct green colonies. *E. coli* cultures fermented glucose and lactose, producing acid and gas; nitrates were not reduced to nitrites, H_2S was not released; did not form urea; but did produce indole.

Cultures of *Klebsiella* spp. are gram-negative nonmotile rods that form capsules. 16 – hourly colonies of *Klebsiella* spp. on solid nutrient media – dome-shaped, 3-4 mm in diameter, with a mucous membrane, on meat peptone agar (MPA) they displayed a grayish-white color; on the Endo medium, the colonies had a pale pink color; on XLD medium – colorless opaque colonies with yellow staining of the medium around the colonies. On HiCrome *E. coli* Agar M 12951 chromogenic medium, selected *Klebsiella* spp. cultures formed transparent, colorless colonies. *Klebsiella* spp. in MPB formed a uniform turbidity of the medium with a stretchy mucous sediment and a film on the surface of the broth culture. They fermented glucose, sucrose, and lactose; did not produce indole or hydrogen sulfide; reduced nitrates to nitrites; exhibited urease activity; and tested positive for lysine and negative for ornithine and phenylalanine.

20 *Enterococcus* spp. cultures were isolated during the studies of the material droppings samples. These gram-positive cocci or ovoids were facultative anaerobes, catalase-negative, and oxidase-negative. They fermented glucose to produce acid without gas, hydrolyzed esculin, and lacked hemolytic activity.

Isolated cultures of *P. aeruginosa* are small homogeneous gram-negative rods (ovoids in appearance, sized 1-5 x 0.5-1.0 microns). They exhibit a consistent turbidity in tryptone-soy broth. On cetrimide agar, they form homogeneous yellow-green, small S-shaped colonies. These bacteria do not produce hydrogen sulfide, do not reduce nitrates, and do not ferment lactose or sucrose. They are capable of producing pyocyanin and demonstrate β-hemolytic activity.

The MPN of isolated groups of microorganisms was determined. In the first experimental group (tab. 1) *E. coli*, *Klebsiella* spp., *Enterococcus* spp. isolated from 10 chickens, whereas *P. aeruginosa* – from 7 chickens.

The MPN index for *E. coli* in chickens of the first group (tab. 1) ranged between $4.6x10^5$–>$1.1x10^7$ colony-forming units (CFU) per gram of sample, with actual counts ranging from $9.0x10^4$ to $4.0x10^7$ CFU/g at a 95% confidence level. The average MPN index for *E. coli* in the samples was $1.0x10^7$ CFU/g.

For *Klebsiella* spp., the MPN index in the first experimental group (tab. 1) ranged from $2.4x10^5$ to $1.1x10^7$ CFU/g, with actual counts ranging from $4.0x10^4$ to $4.0x10^7$ CFU/g at a 95% confidence level. The average MPN index for *Klebsiella* spp. in the samples of chicken droppings was $8.0x10^6$ CFU/g.

The MPN index for *Enterococcus* spp. (tab. 2) in the chicken droppings from the first group ranged from $2.4x10^2$ to $1.1x10^5$ CFU/g, with actual counts ranging from 4.0x10 to $4.0x10^5$ CFU/g at a 95% confidence level. The average MPN index for *Enterococcus* spp. in the samples was $1.8x10^4$ CFU/g.

In the case of *P. aeruginosa* (tab. 2), the MPN index in the litter samples from chickens of the first group ranged from $4.6x10^3$ to $1.1x10^7$ CFU/g, with actual counts ranging from $9.0x10^2$ to $4.0x10^7$ CFU/g at a 95% confidence level. The average MPN index for *P. aeruginosa* was $1.6x10^6$ CFU/g.

The analysis of the obtained data in Tables 1 and 2 showed that the chicken droppings samples differed in terms of species composition of isolated indicator bacteria in chicken droppings (tab. 3). *P. aeruginosa* was isolated in 70% of the samples (samples 1, 4, 5, 6, 8, 9, 10). Given the pathogenic potential associated with this bacterium, the data were categorized into subgroups: A (samples without *P. aeruginosa*) and B (samples where this pathogen was isolated).

Thus, in the samples of subgroup A (samples No. 2, 3, 7), the values of MPN for *E. coli* in all samples

Table 1. Bacteriological examination results (according to the MPN indicator) of chicken droppings samples from group 1.

Samples from chickens, no	Indexes			
	E. coli, [(1)]CFU		*Klebsiella* spp., [(1)] CFU	
	Availability *E.coli,* [(2)] MPN in 1.0 g	The actual number microorganisms per gram within the 95% confidence interval	Availability *Klebsiella* spp., [(2)] MPN in 1.0 g	The actual number microorganisms per gram within the 95% confidence interval
1	$4.6x10^5$	$9.0x10^4$-$1.96x10^6$	>$1.1x10^7$	-
2	>$1.1x10^7$	-	>$1.1x10^7$	-
3	>$1.1x10^7$	-	>$1.1x10^7$	-
4	>$1.1x10^7$	-	>$1.1x10^7$	-
5	>$1.1x10^7$	-	>$1.1x10^7$	-
6	>$1.1x10^7$	-	$2.4x10^5$	$4.0x10^4$-$9.9x10^5$
7	>$1.1x10^7$	-	$2.4x10^5$	$4.0x10^4$-$9.9x10^5$
8	>$1.1x10^7$	-	$2.4x10^6$	$4.0x10^5$-$9.9x10^6$
9	>$1.1x10^7$	-	>$1.1x10^7$	-
10	>$1.1x10^7$	-	>$1.1x10^7$	-
min-max	$4.6x10^5$->$1.1x10^7$	$9.0x10^4$->$4.0x10^7$	$2.4x10^5$->$1.1x10^7$	$4.0x10^4$->$4.0x10^7$
Average value	$1.0x10^7$	-	$8.0x10^6$	-

Notes: (here and further): [(1)] CFU – colony-forming units; [(2)] MPN – most probable number.

Table 2. Results of bacteriological examination (according to the MPN indicator) of chicken litter samples from group 1.

Samples from chickens, No	Indexes			
	P. aeruginosa, [(1)] *CFU*		*Enterococcus* spp., [(1)] CFU	
	Availability *P. aeruginosa* [(2)] **MPN** in 1.0 g	The actual number microorganisms per gram within the 95% confidence interval	Availability *Enterococcus* spp. [(2)] **MPN** in 1.0 g	The actual number microorganisms per gram within the 95% confidence interval
1	$1.5x10^4$	$3.0x10^3$-$3.8x10^4$	$4.6x10^3$	$9.0x10^2$-$1.96x10^4$
2	-	-	$2.4x10^2$	40.0-990.0
3	-	-	$1.1x10^4$	$2.0x10^3$-$4.0x10^4$
4	$1.1x10^5$	$2.0x10^4$-$4.0x10^5$	$1.1x10^5$	$2.0x10^4$-$4.0x10^5$
5	>$1.1x10^7$	-	$1.1x10^4$	$2.0x10^3$-$4.0x10^4$
6	$2.4x10^4$	$4.0x10^3$-$9.9x10^4$	$2.4x10^4$	$4.0x10^3$-$9.9x10^4$
7	-	-	$4.6x10^3$	$9.0x10^2$-$1.96x10^4$
8	$1.1x10^5$	$2.0x10^4$-$4.0x10^5$	$1.1x10^4$	$2.0x10^3$-$4.0x10^4$
9	$4.6x10^3$	$9.0x10^2$-$1.96x10^4$	$1.1x10^3$	200.0-4000.0
10	$1.1x10^4$	$2.0x10^3$-$4.0x10^4$	$2.4x10^3$	$4.0x10^2$-$9.9x10^3$
min-max	$4.6x10^3$>$1.1x10^7$	$9.0x10^2$->$4.0x10^7$	$2.4x10^2$-$1.1x10^5$	40.0-$4.0x10^5$
Average value	$1.6x10^6$	-	$1.8x10^4$	

were within >$1.1x10^7$ CFU/g; indicator of MPN for *Enterococcus* spp. ranged between $2.4x10^2$–$1.1x10^4$ CFU/g, the average value of the indicator was $5.3x10^3$ CFU/g; indicators of NL for *Klebsiella* spp. ranged within $2.4x10^5$–>$1.1x10^7$ CFU/g, the average value of the MPN indicator for *Klebsiella* spp. was $7.4x10^6$ CFU/g.

The content of the indicator bacteria in the sam-

ples of subgroup B was as follows: MPN indicator for *E. coli* ranged from >$1.1x10^7$ CFU/g, with an average of $9.5x10^6$ CFU/g; the low-frequency indicator for *Klebsiella* spp. ranged from $2.4x10^5$ to >$1.1x10^7$ CFU/g, averaging $8.2x10^6$ CFU/g; MPN indicator for *Enterococcus* spp. ranged from $1.1x10^3$ to $1.1x10^5$ CFU/g, averaging $2.3x10^4$ CFU/g; MPN indicator for *P. aeruginosa* ranged from $4.6x10^3$ to >$1.1x10^7$ CFU/g, averaging $1.6x10^6$ CFU/g (tab. 3).

Thus, among the indicator microorganisms, investigated in animal droppings subgroups 1-A and 1-B, *E. coli* exhibited the highest concentration, which was slightly higher in subgroup 1-A than in subgroup 1-B. No significant difference in *Klebsiella* spp. counts was observed between the chicken droppings samples of these subgroups. However, *Enterococcus* spp. counts in subgroup 1-B were consistently higher by 1 lg compared to subgroup 1-A.

Table 3. The content of indicator bacteria in the chicken droppings from group 1.

Indexes	Value	Result, (1) CFU/g	
		group 1 A, n=3	group 1 B, n=7
(2) MPN *E. coli*, (1) CFU/g	D	>$1.1x10^7$	>$1.1x10^7$
	M	>$1.1x10^7$	$9.5x10^6$
(2) MPN *Klebsiellas* spp., (1) CFU/g	D	$2.4x10^5$->$1.1x10^7$	$2.4x10^5$->$1.1x10^7$
	M	$7.4x10^6$	$8.2x10^6$
(2) MPN *Enterococcus* spp., (1) CFU/g	D	$2.4x10^2$-$1.1x10^4$	$1.1x10^3$-$1.1x10^5$
	M	$5.3x10^3$	$2.3x10^4$
(2) MPN *P. aeruginosa.*, (1) CFU/g	D	-	$4.6x10^3$->$1.1x10^7$
	M	-	$1.6x10^6$

Notes (here and further): D – the range of MPN values in the group; M is the average value of MPN in the group.

In bacteriological studies of group 2 chicken droppings from free-range settings (tab. 4), *Klebsiella* spp. and *P. aeruginosa* were not detected, while *E. coli* cultures were isolated in 100% of cases, along *with Enterococcus* spp.

The most probable number (MPN) for *E. coli* in the samples studied ranged from $4.6x10^2$ to $4.6x10^6$ CFU/g, with the actual count of microorganisms ranging from $9.0x10^1$ to $1.96x10^7$ CFU/g at the 95% confidence level. The average MPN value for *E. coli* was $1.4x10^6$ CFU/g.

MPN indicator for *Enterococcus* spp. in samples of litter from chickens from 2 groups ranged from $1.1x10^4$ to >$1.1x10^9$ CFU/g, with the actual count of microorganisms ranging from $2.0x10^3$ to >$1.1x10^9$ CFU/g at the 95% confidence level. The average MPN value for *Enterococcus* spp. was $3.4x10^8$ CFU/g (tab. 4).

In chickens from two groups, the MPN indicator for *Enterococcus* spp. and *E. coli* exceeded 90% of the studied samples: in one sample – by 1 lg (sample No. 9); in 6 samples – by 3 lg (samples No. 1, 2, 4, 6, 7, 8); in 2 samples – by 4 lg (samples No. 5, 10). One sample showed quantitative indicators within one titer (1.1-$2.4x10^4$ CFU/g).

The analysis of the obtained results (tab. 5) showed that, *E. coli, Klebsiellas* spp., *Enterococcus* spp. were isolated from 100% of the samples from chickens kept in simulated industrial poultry house conditions (the first group).

Additionally, *P. aeruginosa*, an insidious causative agent of diseases in both chickens and humans, was isolated from 70% of these samples. *E. coli* and *Enterococcus* spp. were isolated from free-range chickens in 100% of cases. It should be noted that the isolated cultures exhibited cultural, morphological, and biochemical characteristics typical of their species, with no phenotypic signs of dissociation. In group 1 chickens, the average values of MPN indicators were as follows: *E. coli* – $1.0x10^7$ CFU/g; *Klebsiella* spp. – $8.0x10^6$; *Enterococcus* spp. – 1.8x10 CFU/g; *P. aeruginosa* – $1.6x10^6$ CFU/g, while in free-range chickens (group 2) the average value of MPN indicator for *E. coli* was $1.4x10^6$ CFU/g (i.e. lower by 1 lg); the average value of the MPN indicator for *Enterococcus* spp. – $3.4x10^8$ CFU/g (i.e. higher by 4 lg). Notably, in free-range chickens, no potentially pathogenic *Klebsiella* spp. or *P. aeruginosa* were found; however, there was a significantly higher presence of *Enterococcus* spp., which is part of the normal intestinal microflora of chickens.

Table 4. Results of bacteriological examination (according to the MPN indicator) of chicken droppings samples from group 2.

Samples from chickens, no	Indexes			
	E. coli, (1) CFU		*Enterococcus* spp., (1) CFU	
	Availability *E. coli* (2) MPN in 1.0 g	The actual number microorganisms per gram within the 95% confidence interval	Availability *Enterococcus* spp. (2) MPN in 1.0 g	The actual number microorganisms per gram within the 95% confidence interval
1	$4.6x10^6$	$9.0x10^5$-$1.96x10^7$	>$1.1x10^9$	-
2	$4.6x10^2$	$9.0x10^1$-$1.96x10^3$	$1.1x10^5$	$2.0x10^4$-$4.0x10^5$
3	$2.4x10^4$	$4.0x10^3$-$9.9x10^4$	$1.1x10^4$	$2.0x10^3$-$4.0 x10^4$
4	$4.6x10^6$	$9.0x10^5$-$1.96x10^7$	>$1.1x10^9$	-
5	$4.6x10^4$	$9.0x10^3$-$1.96 x10^5$	$1.1x10^8$	$2.0x10^7$-$4.0x10^8$
6	$4.6x10^6$	$9.0x10^5$-$1.96x10^7$	>$1.1x10^9$	-
7	$4.6x10^3$	$9.0x10^2$-$1.96x10^4$	$1.1x10^6$	$2.0x10^5$-$4.0x10^6$
8	$2.4x10^3$	$4.0x10^2$-$9.9x10^4$	$1.1x10^6$	$2.0x10^5$-$4.0x10^6$
9	$2.4x10^4$	$4.0x10^3$-$9.9x10^4$	$4.6x10^5$	$9.0x10^4$-$1.96x10^6$
10	$2.4x10^3$	$4.0x10^2$-$9.9x10^3$	$2.4x10^7$	$4.0x10^6$-$9.9x10^9$
min-max	$4.6x10^2$-$4.6x10^6$	$9.0x10^1$-$1.96x10^7$	$1.1x10^4$->$1.1x10^9$	$2.0x10^3$->$1.1x10^9$
Average value	$1.4x10^6$	-	$3.4x10^8$	-

Table 5. The content of indicator bacteria in the droppings of chickens from both groups, 1 and 2.

Indexes	Value	Result, (1) CFU/g	
		group 1 A, n=10	group 1 A, n=10
(2) MPN *E. coli*, (1) CFU/g	D	$4.6x10^5$->$1.1x10^7$	$4.6x10^2$-$4.6x10^6$
	M	**$1.0x10^7$**	**$1.4x10^6$**
(2) MPN *Klebsiellas* spp., (1) CFU/g	D	$2.4x10^5$->$1.1x10^7$	-
	M	$8.0x10^6$	-
(2) MPN *Enterococcus* spp., (1) CFU/g	D	$2.4x10^2$-$1.1x10^5$	$2.0x10^3$->$1.1x10^9$
	M	**$1.8x10^4$**	**$3.4x10^8$**
(2) MPN *P. aeruginosa*, (1) CFU/g	D	$4.6x10^3$->$1.1x10^7$	-
	M	$1.6x10^6$	-

DISSCUTIONS

This study focused on identifying differences in the content of indicator microorganisms. According to current concepts of risk assessment for habitats and ecosystems, various biological entities, including microorganisms (viruses, bacteriophages, bacteria, fungi), helminth eggs, microscopic algae, and a range of protozoa, can be used as indicators. Currently, certain bacteria are used to assess the status of water bodies (27 – 31). The presence of indicator microorganisms in a specific ecological or biological niche may signal the presence of other pathogens (32 – 34). For example, the bacteriophage *Bacteroides fragilis* has been proposed as a potential indicator of human viruses in the environment.

Common coliforms, fecal coliforms, *E. coli*, and enterococci are more often used as indicators of anthropogenic pressure on sources of drinking water.

Additionally, assessing fecal contamination of surface and drinking water using groups of microorganisms is quite common., e.g. – *Escherichia*, *Citrobacter*, *Enterobacter.* The following should be identified among possible indicator bacteria: *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Mycobacterium tuberculosis*, *Streptococcus bovis*, *Naegleria fowleri*, *Legionella* spp., *Mycobacterium*

spp., *Aeromonas* spp., *Acanthamoeba* spp., *Staphylococcus* spp., *Legionella* spp., *Salmonella* spp., *Shigella* spp., *Clostridium* spp., *Legionella* spp., *Yersinia* spp., *Campylobacter* spp., *Listeria* spp., yeast, etc. (35).

Identifying these indicator microorganisms and determining their sources of spread is crucial for controlling the risks of disease outbreaks, particularly those of zoonotic origin. This goal can be achieved by determining the phenotypic characteristics of the microorganisms or their genetic markers.

In recent years, methods for quantitatively determining specific segments of DNA or RNA in the genome, such as PCR and next-generation sequencing (NGS), have been employed to identify genetic markers (36 – 40). The source of contamination is identified based on the analysis of genetic sequences unique to both the specific microorganism and the host organism from which it originates.

Phenotypic characteristics of indicator microorganisms are assessed using traditional bacteriological methods. When performing these studies, the test for determining the most probable number of microorganisms (MPN, NCH) was used to detect not only the presence of certain genera of bacteria, but also to estimate their number (41). The results obtained regarding the presence of indicator bacteria in chicken litter under different housing conditions support the significant influence of the macroorganism habitat on the microbiome's species composition (42 – 47).

CONCLUSIONS

1. The research results revealed a difference in the composition of indicator bacteria in chicken droppings under various keeping conditions. Chickens raised in backyard settings showed no presence of zoonotic bacteria with pathogenic potential, compared to chickens kept under artificially controlled optimal climatic conditions and fed on a standard diet.
2. The obtained results provide the basis for an in-depth study of the microbiome in the digestive tract of chickens under different keeping conditions. Additionally, they aim to elucidate the mechanisms of microbiome formation and influence, with the goal of improving the technology for producing safe, high-quality poultry products in backyard farms and developing effective recommendations to ensure proper bioprotection levels for poultry maintained for personal consumption.

CONFLICT OF INTEREST

All authors declare no competing interests.

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REFERENCES

1. Shi N, Li N, Duan X, Niu H. Interaction between the gut microbiome and mucosal immune system. *Mil Med Res*. 2017;4:14. doi:10.1186/s40779-017-0122-9
2. Li J, Li Y, Xiao H, et al. The intestinal microflora diversity of aboriginal chickens in Jiangxi province, China. *Poult Sci*. 2024;103(2):103198. doi:10.1016/j.psj.2023.103198
3. Thomas M, Wongkuna S, Ghimire S, et al. Gut Microbial Dynamics during Conventionalization of Germfree Chicken. *mSphere*. 2019; 4(2):e00035-19. doi:10.1128/mSphere.00035-19
4. Bensch HM, Lundin D, Tolf C, Waldenström J, Zöttl M. Environmental effects rather than relatedness determine gut microbiome similarity in a social mammal [published correction appears in J Evol Biol. 2024 May 6;37(5):577-578]. *J Evol Biol*. 2023;36(12):1753-1760. doi:10.1111/jeb.14208
5. Stanley D, Hughes RJ, Moore RJ. Microbiota of the chicken gastrointestinal tract: influence on health, productivity and disease. *Appl Microbiol Biotechnol*. 2014;98(10):4301-4310. doi:10.1007/s00253-014-5646-2
6. Chen CY, Chen CK, Chen YY, et al. Maternal gut microbes shape the early-life assembly of gut microbiota in passerine chicks via nests. *Microbiome*. 2020;8(1):129. doi:10.1186/s40168-020-00896-9
7. Olsen R, Kudirkiene E, Thøfner I, et al. Impact of egg disinfection of hatching eggs on the eggshell microbiome and bacterial load. *Poult Sci*. 2017; 96(11):3901-3911. doi:10.3382/ps/pex182
8. Fathima S, Shanmugasundaram R, Adams D, Sel

varaj RK. Gastrointestinal Microbiota and Their
9. Manipulation for Improved Growth and Performance in Chickens. *Foods*. 2022;11(10): 1401. doi:10.3390/foods11101401
10. Turko I, Ushkalov V. Biofilm-forming ability of coccus forms of the caecal microflora of laying hens when using the probiotic and nanonutrition cobalt. *Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies. Series: Veterinary Sciences.* 2018;20(87):60-64. doi:org/10.15421/nvlvet8712
11. David LA, Maurice CF, Carmody RN, et al. Diet rapidly and reproducibly alters the human gut microbiome. *Nature*. 2014;505(7484):559-563. doi:10.1038/nature12820
12. Zhou X, Stephens M. Genome-wide efficient mixed-model analysis for association studies. *Nat Genet*. 2012;44(7):821-824. doi:10.1038/ng.2310
13. Chen B, Li D, Leng D, Kui H, Bai X, Wang T. Gut microbiota and meat quality. *Front Microbiol*. 2022; 13:951726. doi:10.3389/fmicb.2022.951726
14. Conte G, Dimauro C, Daghio M, et al. Exploring the relationship between bacterial genera and lipid metabolism in bovine rumen. *Animal*. 2022; 16(5):100520. doi:10.1016/j.animal.2022.100520
15. Videnska P, Faldynova M, Juricova H, et al. Chicken faecal microbiota and disturbances induced by single or repeated therapy with tetracycline and streptomycin. *BMC Vet Res*. 2013;9:30. doi:10.1186/1746-6148-9-30
16. Subirats J, Murray R, Scott A, Lau CH, Topp E. Composting of chicken litter from commercial broiler farms reduces the abundance of viable enteric bacteria, Firmicutes, and selected antibiotic resistance genes. *Sci Total Environ*. 2020;746: 141113. doi:10.1016/j.scitotenv.2020.141113
17. Hinton A Jr, Buhr RJ, Ingram KD. Physical, chemical, and microbiological changes in the ceca of broiler chickens subjected to incremental feed withdrawal. *Poult Sci*. 2000;79(4):483-488. doi:10.1093/ps/79.4.483
18. Zoetendal EG, Rajilic-Stojanovic M, de Vos WM. High-throughput diversity and functionality analysis of the gastrointestinal tract microbiota. *Gut*. 2008;57(11):1605-1615. doi:10.1136/gut.2007.133603
19. Gong, J., Si, W., Forster, R.J., Huang, R., Yu, H., Yin, Y., et al. 16S rRNA gene-based analysis of mucosa-associated bacterial community and phylogeny in 363the chicken gastrointestinal tracts: From crops to ceca. *FEMS Microbiol. Ecol.* 2007;*59*(1): 147-157. doi:10.1111/j.1574-6941.2006.00193.x
20. Gong J, Forster RJ, Yu H, et al. Diversity and phylogenetic analysis of bacteria in the mucosa of chicken ceca and comparison with bacteria in the cecal lumen. *FEMS Microbiol Lett*. 2002;208(1):1-7. doi:10.1111/j.1574-6968.2002.tb11051.x
21. Wei S, Morrison M, Yu Z. Bacterial census of poultry intestinal microbiome. *Poult Sci*. 2013;92(3): 671-683. doi:10.3382/ps.2012-02822
22. Apajalahti J, Kettunen A, Graham H. Characteristics of the gastrointestinal microbial communities, with special reference to the chicken. *Worlds Poult. Sci. J.* 2004;60:223–232. doi:10.1079/WPS20040017
23. Aruwa CE, Pillay C, Nyaga MM, Sabiu S. Poultry gut health - microbiome functions, environmental impacts, microbiome engineering and advancements in characterization technologies. *J Anim Sci Biotechnol*. 2021;12(1):119. doi:10.1186/s40104-021-00640-9
24. Oakley BB, Lillehoj HS, Kogut MH, et al. The chicken gastrointestinal microbiome. *FEMS Microbiol Lett*. 2014;360(2):100-112. doi:10.1111/1574-6968.12608
25. Kamada N, Seo SU, Chen GY, Núñez G. Role of the gut microbiota in immunity and inflammatory disease. *Nat Rev Immunol*. 2013;13(5):321-335. doi:10.1038/nri3430
26. Sommer F, Bäckhed F. The gut microbiota-masters of host development and physiology. *Nat Rev Microbiol*. 2013;11(4):227-238. doi:10.1038/nrmicro2974
27. Kogut MH. The effect of microbiome modulation on the intestinal health of poultry. *Anim Feed Sci Technol.* 2019;250:32–40. doi:10.1016/j.anifeedsci.2018.10.008
28. Gerba C. Indicator Microorganisms. In: *Environmental Microbiology*. Maier R, Pepper I, Gerba C. (eds). Academic Press, New York, 2000. Available at: https://bly.covenantuniversity.edu.ng/ebooks/Environmental_Microbiology/Chapter-23---Indicator-Microorganisms_2015_Environmental-Microbiology.pdf [Accessed May 25, 2024].
29. Wen X, Chen F, Lin Y, Zhu H, Yuan F, Kuang D, Jia Z, Yuan Z. Microbial Indicators and Their Use for Monitoring Drinking Water Quality-A Review. *Sustainability*. 2020;12(6):2249. doi:org/10.3390/su12062249
30. Jung B, Hoilat GJ. MacConkey Medium. In: StatPearls. Treasure Island (FL): StatPearls, 2024. Available from: https://www.ncbi.nlm.nih.gov/books/NBK557394/ [Accessed May 26, 2024].
31. Bahrndorff S, Alemu T, Alemneh T, Lund Nielsen J. The Microbiome of Animals: Implications for Conservation Biology. *Int J Genomics*. 2016;2016: 5304028. doi:10.1155/2016/5304028
32. Noble RT, Moore DF, Leecaster MK, McGee CD, Weisberg SB. Comparison of total coliform, fecal coliform, and enterococcus bacterial indicator response for ocean recreational water quality testing. *Water Res*. 2003;37(7):1637-1643. doi:10.1016/S0043-1354(02)00496-7

33. Dhivahar J, Parthasarathy A, Krishnan K, Kovi BS, Pandian GN. Bat-associated microbes: Opportunities and perils, an overview. *Heliyon*. 2023; 9(12):e22351. doi:10.1016/j.heliyon.2023.e22351
34. Motlagh AM, Yang Z. Detection and occurrence of indicator organisms and pathogens. *Water Environ Res*. 2019;91(10):1402-1408. doi:10.1002/wer.1238
35. Lei B, Xu Y, Lei Y, Li C, Zhou P, Wang L, Yang Q, Li X, Li F, Liu C, Cui C, Chen T, Ni W, Hu S. CRAMdb: a comprehensive database for composition and roles of microbiome in animals. *Nucleic Acids Res*. 2023;51(D1):D700-D707. doi:10.1093/nar/gkac973
36. Levin D, Raab N, Pinto Y, et al. Diversity and functional landscapes in the microbiota of animals in the wild. *Science*. 2021;372(6539):eabb5352. doi:10.1126/science.abb5352
37. Wang X, Tsai T, Deng F, et al. Longitudinal investigation of the swine gut microbiome from birth to market reveals stage and growth performance associated bacteria.*Microbiome*. 2019;7(1):109. doi:10.1186/s40168-019-0721-7
38. Xu Y, Lei B, Zhang Q, et al. ADDAGMA: A database for domestic animal gut microbiome atlas. *Comput Struct Biotechnol J*. 2022;20:891-898. doi:10.1016/j.csbj.2022.02.003
39. Hu R, Li F, Chen Y, et al. AnimalMetaOmics: a multi-omics data resources for exploring animal microbial genomes and microbiomes. *Nucleic Acids Res*. 2024;52(D1):D690-D700. doi:10.1093/nar/gkad931
40. Qin D. Next-generation sequencing and its clinical application. *Cancer Biol Med*. 2019;16(1):4-10. doi:10.20892/j.issn.2095-3941.2018.0055
41. Scallan E, Hoekstra RM, Angulo FJ, et al. Foodborne illness acquired in the United States--major pathogens. *Emerg Infect Dis*. 2011;17(1):7-15. doi:10.3201/eid1701.p11101
42. Schwaiger K, Schmied EM, Bauer J. Comparative analysis of antibiotic resistance characteristics of Gram-negative bacteria isolated from laying hens and eggs in conventional and organic keeping systems in Bavaria, Germany. *Zoonoses Public Health*. 2008;55(7):331-341. doi:10.1111/j.1863-2378.2008.01151.x
43. Ortega C, Solo-Gabriele HM, Abdelzaher A, Wright M, Deng Y, Stark LM. Correlations between microbial indicators, pathogens, and environmental factors in a subtropical estuary. *Mar Pollut Bull*. 2009;58(9):1374-1381. doi:10.1016/j.marpolbul.2009.04.015
44. Grunkemeyer VL. Zoonoses, public health, and the backyard poultry flock. *Vet Clin North Am Exot Anim Pract*. 2011;14(3):477-vi. doi:10.1016/j.cvex.2011.05.010
45. Rajkumar U, Rama Rao SV, Raju MVLN, Chatterjee RN. Backyard poultry farming for sustained production and enhanced nutritional and livelihood security with special reference to India: a review. *Trop Anim Health Prod*. 2021;53(1):176. doi:10.1007/s11250-021-02621-6
46. Chaiban C, Robinson TP, Fèvre EM, et al. Early intensification of backyard poultry systems in the tropics: a case study. *Animal*. 2020;14(11):2387-2396. doi:10.1017/S175173112000110X
47. Derksen T, Lampron R, Hauck R, Pitesky M, Gallardo RA. Biosecurity Assessment and Seroprevalence of Respiratory Diseases in Backyard Poultry Flocks Located Close to and Far from Commercial Premises. *Avian Dis*. 2018; 62(1):1-5. doi:10.1637/11672-050917-Reg.1
48. Xiao J, Cheng Y, Zhang W, Lu Q, Guo Y, Hu Q, Wen G, Shao H, Luo Q, Zhang T. Genetic characteristics, antimicrobial susceptibility, and virulence genes distribution of *Campylobacter* isolated from local dual-purpose chickens in central China. *Front Cell Infect Microbiol*. 2023;13:1236777. doi: 10.3389/fcimb.2023.1236777

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